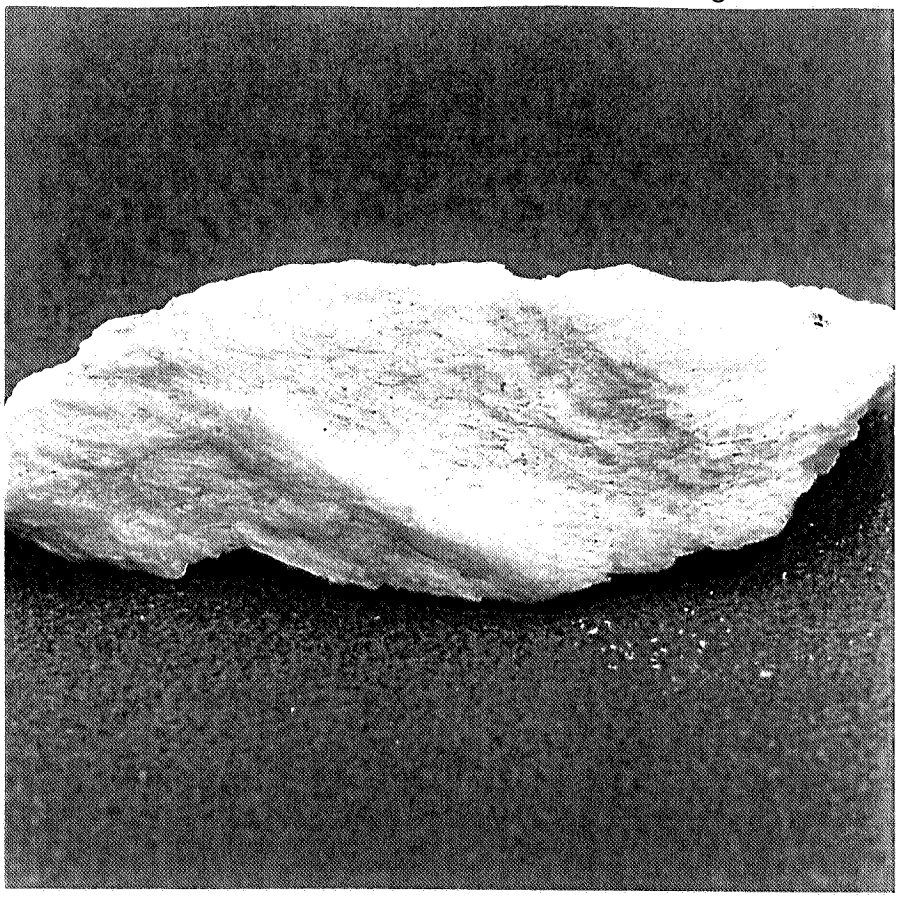


Plate 8: Macroscopic sample of tremolite from :- Ochsenfeld, Binntal. Switzerland.

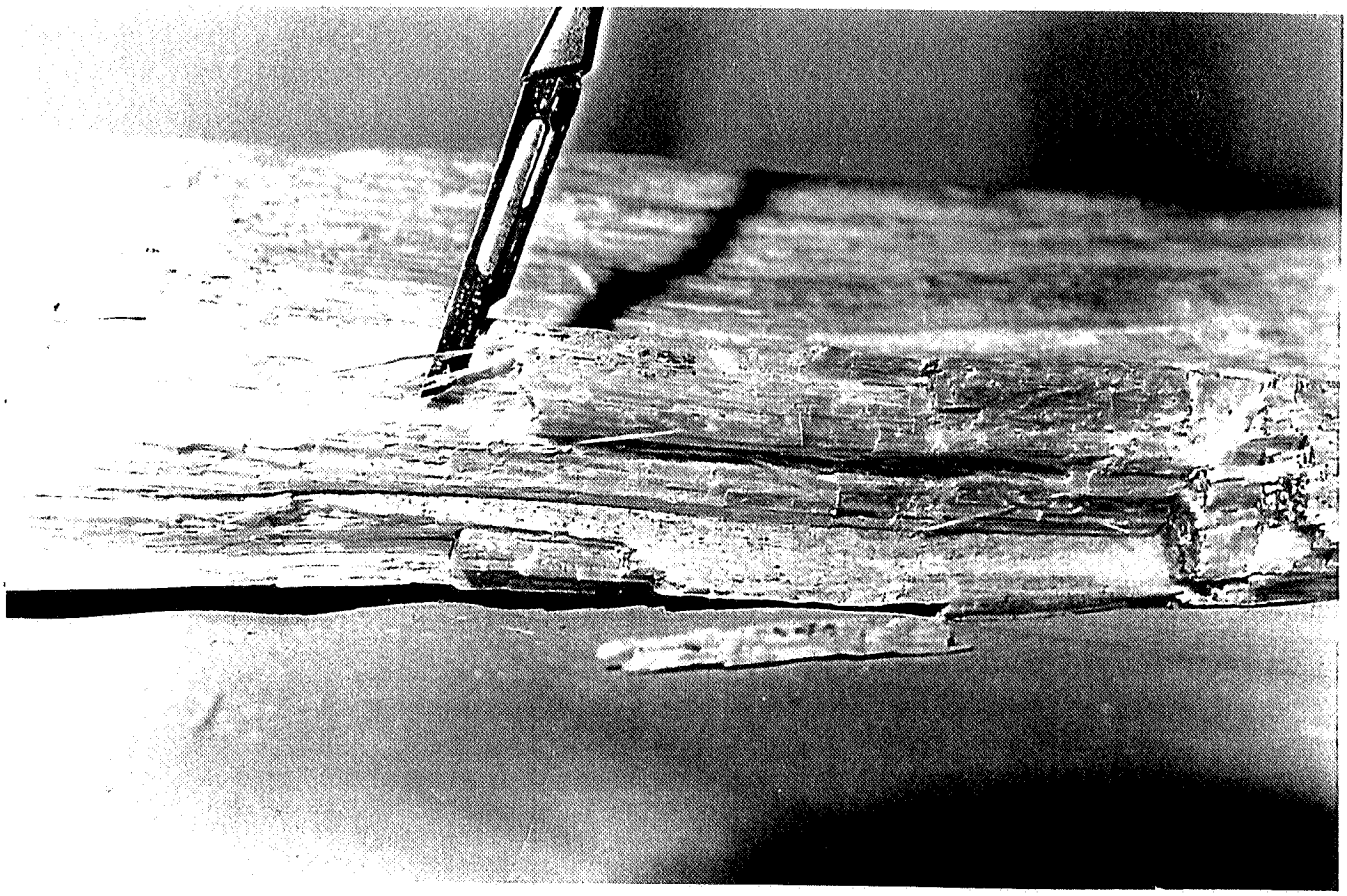


Plate 9: Macroscopic sample of tremolite from :- North European dolomites

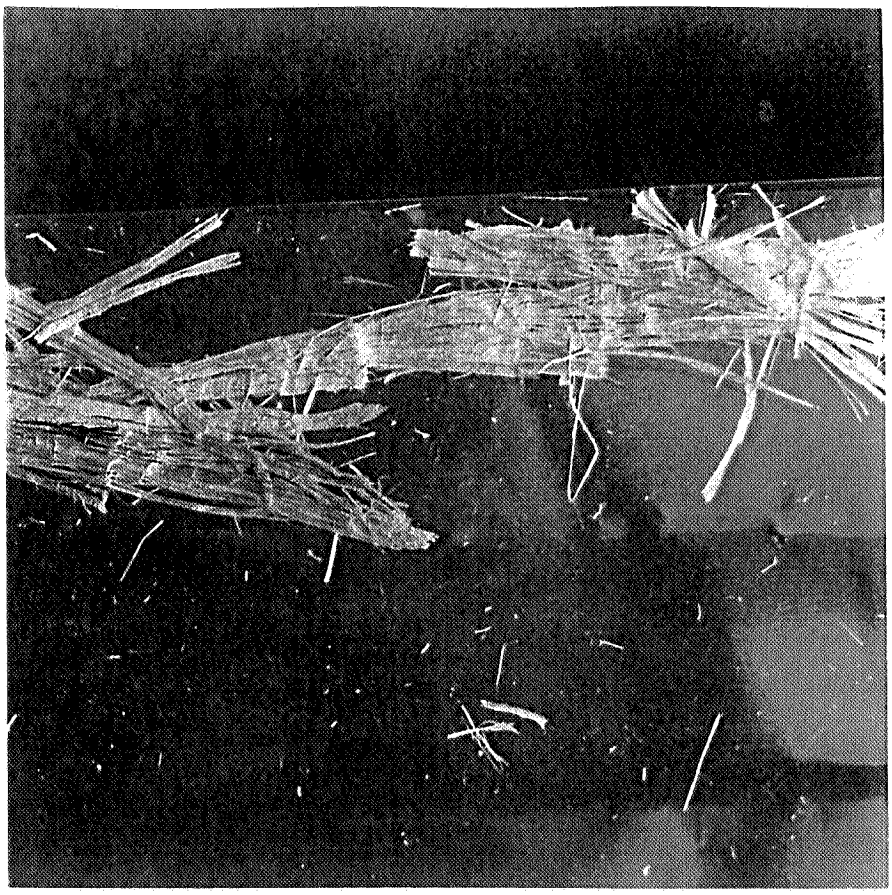


Plate 10: Macroscopic sample of tremolite from :- Glenealy, Co Wicklow.

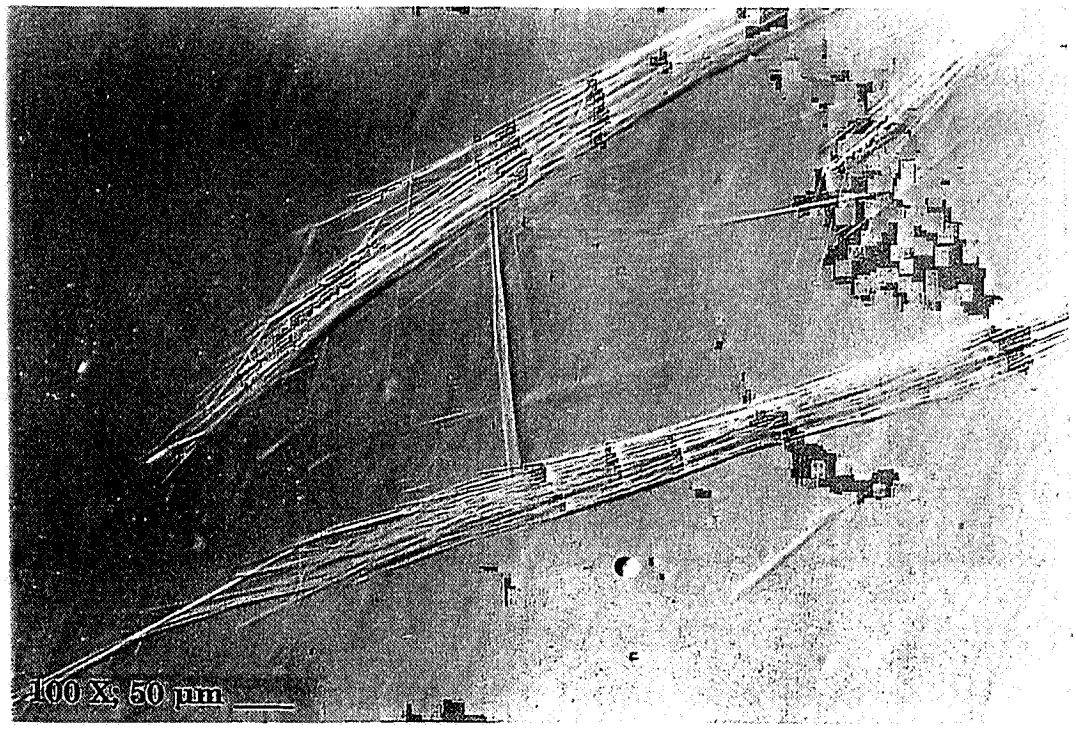


Plate 11: Interference optical microscopy image of tremolite asbestos, Jamestown, California.

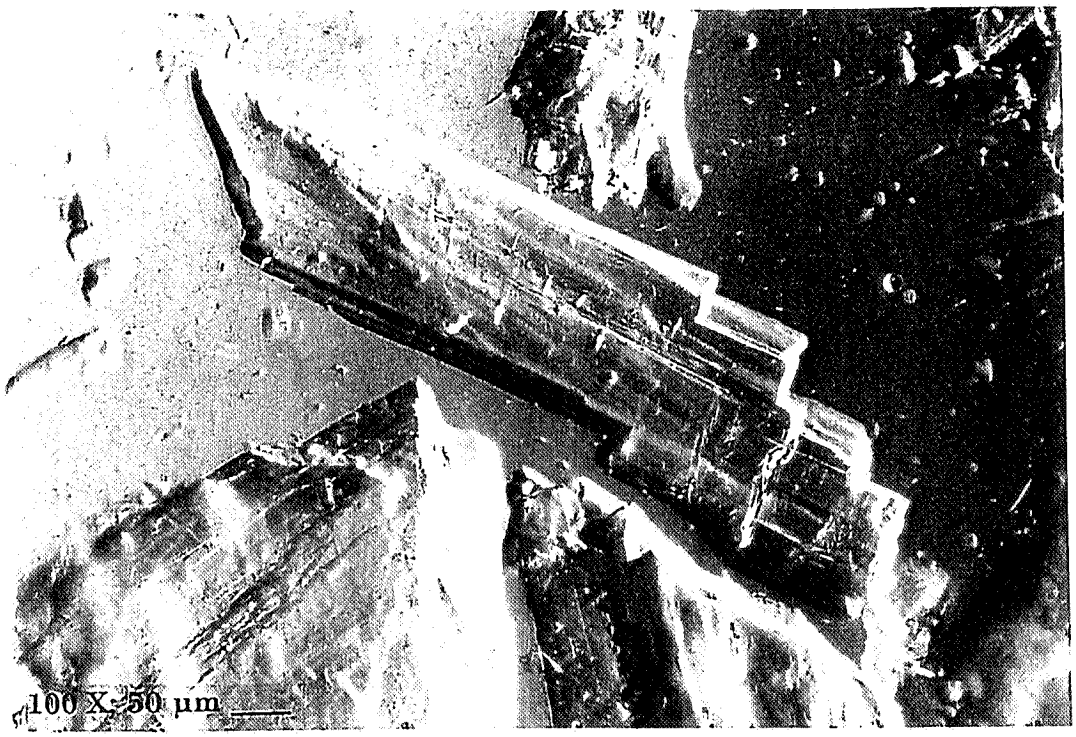


Plate 12: Interference optical microscopy image of tremolite, Shininess, Lairg.

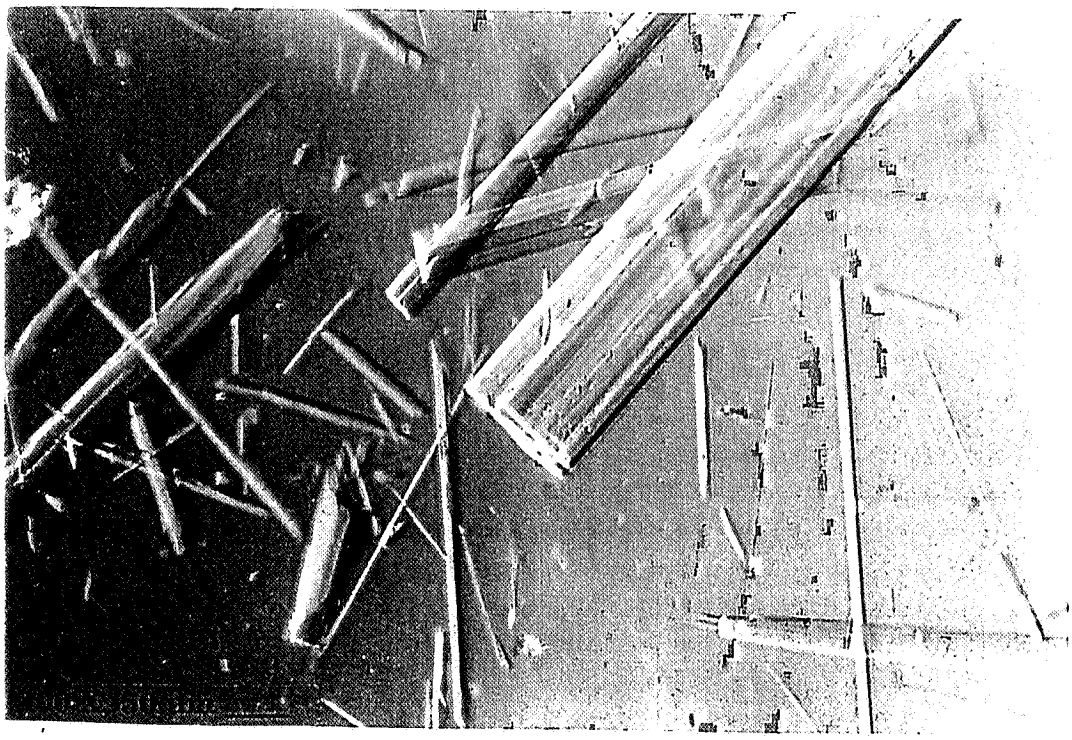


Plate 13: Interference optical microscopy image of tremolite, Ala de Stura, Italy.

As seen below in figure 2 indian tremolite did not conform to the hypothesis that there is a distinct discrimination between asbestos and non asbestos mineral fragments as it gave a slope between the two groups that had been chosen for study.

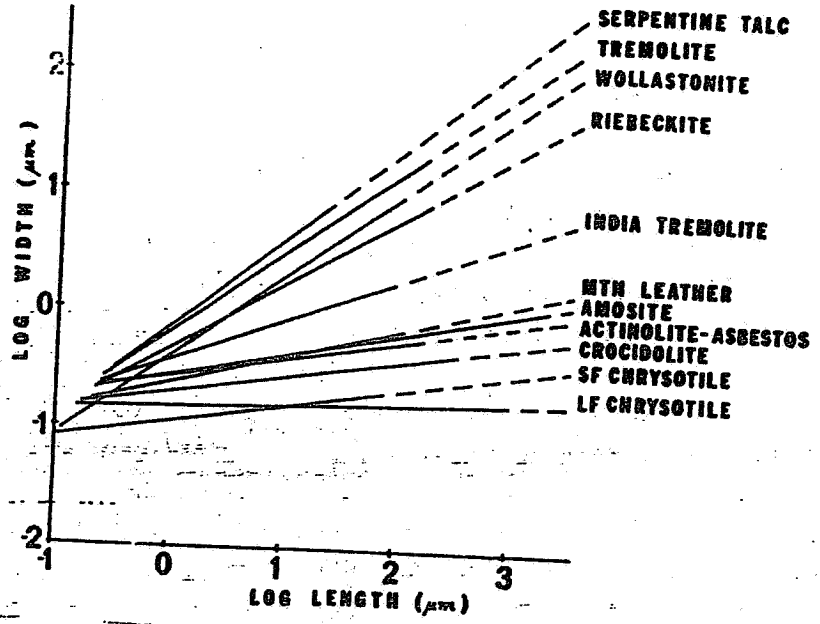


Figure 2: Least squares linear regression plots of log width as a function of log length from Wylie and Schweitzer (1982).

Chatfield and Dillon (1982) used the median (M_{AR}) and geometric standard deviation (GSD_{AR}) of the aspect ratio distribution to give an index of fibrosity (F) based on the value of M_{AR} raised to the power of the GSD_{AR}. They found that for water suspensions of fibres (> 0.5 μm long) a value of greater than 50 was usually achieved by asbestos populations.

In many mineralogical samples where asbestos is a contaminant, particles with habits in the continuum between asbestos and equant are likely to be common. This means that the simple asbestos/non-asbestos classification sought by discriminant analysis may be an unrealistic oversimplification and it is common to have elongated mineral fragments contaminated with asbestos fibres. By careful inspection of fibre morphology and the size distributions, it is arguable that it is possible to choose a length, width or aspect ratio discriminant, which will give approximately the right answer for the population (i.e. some short asbestos fibres or low aspect ratio bundles of asbestos are incorrectly classified as non-asbestos but this is balanced by a similar number of non-asbestos fibres who have high aspect ratios and are presumed to be asbestos). This decision point is to some extent intuitive and to an extent discriminant analysis will help locate and justify the parameters used. However, this will only work well on samples where the overlap between the size parameters is relatively small and a detailed analysis of the size distribution has been obtained. Samples from different

geological locations and subject to different sampling and grinding procedures will require a reassessment of the criterea for discrimination. However, there is much evidence that the 3:1 aspect ratio used for airborne fibre analysis, is not an appropriate discriminator to apply for the analysis of bulk asbestos content.

4.2.4 *Published methods for discriminating cleavage fragments*

A number of methods have been published since the project started which go some way to assessing and quantifying between asbestos fibres and cleavage fragments.

The ID -191 method (OSHA, 1994) allows light microscopy discrimination of cleavage fragments with widths >1μm showing oblique extinction. For analysis of fibres of all widths SEM or TEM examination was recommended, but no method for determining between cleavage fragments and asbestos fibres using these two methods was published.

MDHS 77 allows for PLM characterisation of the fibre population based on extinction angle and population characteristics and MDHS 87 gives more detailed methods for fibre discrimination on a fibre -by -fibre basis by PCM/PLM observations and gives guidelines for SEM and TEM analysis of asbestos fibres for further determination of the fine fibre content.

A Nordic method has been developed for the analysis of tremolite in dolomites and the sample preparation strategy for this method has been incorporated in the EU method developed for this project. An improved optical method using microscopy and image analysis to count and size the fibres was reported by Lungren et al. 1996. This method is also interesting in that it mounts the samples on cellulose nitrate filters using cimaldehyde to produce a blue dispersion staining colour in the wider tremolitic fibres, making it possible to exclude other non-tremolitic mineral fragments more easily. Unfortunately it does not produce permanent samples.

Recent investigations of Finnish quarries (Hartikainen,and Tossavainen, 1997) have attempted to discriminate regulatory fibres into three categories: perfect fibres, cleavage fragments and an intermediate group showing both characteristics called fibrous cleavage fragments; on the basis of their morphology in the SEM. They found that aspect ratios varied between the three groups, but as aspect ratio is such an important part of the morphological discrimination, the two are not independent. However, they estimated that an aspect ratio of 5:1 and 10:1 would exclude 39 and 95% of the cleavage fragments and 5 and 61% of the fibrous cleavage fragments and 3 and 21% of the perfect fibres in their samples. Other authors have reported similar ratios (Campbell et al., 1977, 1979, Wylie, 1988).

4.3 Relative potency of asbestos and non-asbestos fibres. - Is discrimination justified on health grounds?

4.3.1 *Initial reasons for inclusion of non-asbestiform actinolite, tremolite and anthophyllite*

OSHA in its original rule making in 1972 included non-asbestiform fibres of anthophyllite, actinolite and tremolite because of the emerging results from a series of animal implantation experiments. The work by two independent groups Stanton and Wrench (1972) and Pott and Friedrichs (1972) produced the following conclusions:

1) "That the durable fibrous shape of asbestos particles is the true property of their carcinogenic effect in humans.

2) Chemical components and surface properties are not the decisive carcinogenic agents.

3) Elongated particles generally may have a carcinogenic potential as well as asbestos fibres if they are sufficiently long thin and durable."

Both groups went on to refine their experiments to produce data to link carcinogenic potential to fibre dimensions (Stanton et al. 1981; Pott, 1993) which remain essentially unchallenged (although frequently reinterpreted , e.g Wylie et al., 1993) to this day.

4.3.2 *Epidemiology*

There is epidemiological evidence that tremolite asbestos is a potent human carcinogen both as a contaminant in mining environments (Mc Donald et al. 1986; Amandus et al. 1987. Mc Donald, 1997) and from environmental exposures (Baris et al. 1988; Yazicioglu et al., 1980; Langer et al., 1977). There is similar evidence that non-asbestiform tremolite (Gamble et al. 1982) and cummingtonite-grunerite (Mc Donald et al. 1978, Higgins et al. 1983) does not produce increased mortality. However there are also studies which initially suggested a possible increase in lung cancers due to exposure to non-asbestiform tremolitic talc (Dement and Zumwalde, 1977; Dement et al. 1980) but this evidence was vigorously debated in the OSHA 1992 rule making and was influential in deciding that there was a 'insufficient epidemiological evidence to inform as to the carcinogenicity of non-asbestiform fibres. The epidemiological evidence available from studies of non- asbestiform fibres, was however limited by low respirable fibre counts and short latency periods, so an excess standardised mortality ratio (SMR) would be highly unlikely to be observed. Therefore the OSHA interpretation of the epidemiology is highly disputable, but OSHA rulemaking (after legal challenges) is burdened with proving the health findings and cannot take a prudent stance.

4.3.3 *Reviews of fibre toxicology since 1990*

Although much of the evidence is described in the published rulemaking (OSHA, 1992), a review by the American Thoracic Society (ATS, 1990) gave a lengthy review of the data available at the time. However, the conclusions of the ATS review did not

accord with the Industry or OSHA's pre-published intention to remove the non-asbestiform minerals from the rule, 'At present, the prudent public health policy course is to regard appropriately sized tremolite fibres in sufficient exposure and dose (concentration and duration) as capable of producing the recognised asbestos related diseases and should be regulated accordingly'. Alternative reviews, many industry funded (NSA, 1990, Nolan et al. 1991, Reger and Morgan, 1990),disagreed with the ATS conclusions. For example an editorial looking at the same data concluded, 'that there was no evidence to suggest than non-asbestiform mineral counterparts of asbestos are carcinogens in animals' and mortality studies, 'argue convincingly against a casual connection between lung cancer and non-asbestiform tremolite'. Although at first sight the two articles and sides seem to be mutually exclusive, it can be seen that both are essentially drawing the same conclusions, if there is agreement on what are the appropriate dimensions for non-asbestiform tremolite.

An review of fibre toxicology (Meldrum, 1995) concluded that the current definitions of regulatory fibres for length and width are justified by the available toxicological data and should not be changed. However it was noted "that there is good evidence from animal studies to suggest that short fibres (<5 μm long) pose little if any concern for disease development at any site; fibres of lengths at least in the region of 10-15μm are necessary to produce disease in the lungs; but shorter fibres in the region of 8-10 μm can cause mesothelioma. There is no biological reason to suppose that a sharp cut-off value in fibre length would separate hazardous from non-hazardous fibres. This suggests that there would be no justification for increasing the current value of 5μm as the length of the 'regulated' fibre". The review found little evidence available on the role of fibre diameter noting that, " fine fibres may appear more toxic due to their greater efficiency of lung deposition following inhalation exposure. There is no evidence that thinner fibres are more toxic than thicker fibres at a cellular level, when comparisons are based on numbers of fibres". The review made no recommendations on aspect ratio merely saying, "that there is a lack of specific toxicological evidence". Although some of these conclusions may be regarded as highly questionable by some (e.g. Wylie,1984; Kelse and Thompson, 1989; Wylie, 1990; NSA, 1990; Wylie and Bailey, 1992;) this review was published by HSE to represent, " HSE's stance on fibre toxicology".

4.3.4 *Animal data*

Stanton et al. (1981), Smith (1974 and 1979), Wagner et al. (1982) and Davies et al. (1992) have shown that non-asbestiform and asbestiform tremolites have wide differences in carcinogenic potency, when compared in animal experiments. A later analysis (ASA, 1990) of the size distribution of the fibres used by the researchers' showed that change in potency correlated with the numbers of > 5 μm long and < 1 μm width fibres used. This is therefore in agreement with the results from implantation/injection tests on rats using other types of asbestos fibres.

The recent MMVF animal inhalation data, available from MMVF industry funded studies at the Research and Consulting Company (RCC) Geneva (Bunn et al. 1994) and from the variously funded COLT programme (Jones et al. 1997), allow important comparisons between asbestos and non- asbestos fibres to be made. These studies essentially further confirmed, that given sufficient durability it is the dimensions and dose which will determine the hazard and risk. However, as the exposure, dose and

durability of MMVF's used in animal experiments can be accurately measured, the fibre size effects on toxicity can be studied. Furthermore, as asbestos controls were used in these experiments (with much greater exposures by fibre number (>5 μm long fibres) compared to the MMVF's) it is also possible to conclude that on a fibre by fibre basis the larger MMVF fibres (which had durability half -lives of a year or more in the rat lung) were many times more toxic than asbestos fibres. The typical geometric mean size distributions for the MMVF fibres were 0.8 - 1.0 μm width and 15 - 18 μm length. These fibres were considerably wider than the asbestos fibres but were considered to be *rat* respirable.

4.3.5 *Application of animal data to cleavage fragments*

If the results of the MMVF experiments are applied to the asbestos - cleavage fragment debate it is apparent that the chemical composition is important only in terms of fibre durability. The chemical durability of the same mineral in two different morphological types is likely only to differ by the ratio of exposed surface areas. This is probably not a significant difference, as the chemical durability half life of asbestos is of the order of tens of years. In terms of shape and density there is unlikely to be any difference in the respirability or retention of the different fibrous habits if of *human* respirable size (<3 μm width). Therefore fibre dimensions would appear to offer the only scientific reason for any toxicological differences between elongated cleavage fragments and asbestos. It is consistent to expect little toxicity for the short fibres (<5 μm long) distributions, so differences in the dimensions of the longer fibres offers the only possible reason for differences in the hazard and risk.

4.3.6 *Exposure and dose considerations*

Animal inhalation experiments as well as human exposure measurements generally consider that the exposure is equivalent to dose. However this not the case for a number of reasons when considering the health effects of asbestos and cleavage fragment.

One major difference is that asbestos fibres may consist of bundles of fine fibrils which may divide longitudinally in the lung producing a dose of long thin fibres greater than the measured fibre exposure. Different types of fibre may not have the same ease of opening (dividing longitudinally). Fibril sizes may vary widely (0.01 - 1 μm width) between different amphibole types and amphibole fibres of different origin. Crocidolite generally has the finest fibrils and anthophyllite the coarsest. There is little visible oir actual difference between a 10 x 0.5 μm macroscopic fibre of anthophyllite and a 10 x 0.5 μm cleavage fragment of the same mineral. For a comparable fibre of crocidolite and cleavage fragment of riebeckite, there is a significant difference: crocidolite has an average fibril width of 0.03 μm which give it the capacity to divide into about 300 finer fibrils in the lung. A MMVF fibre and a single crystal elongated cleavage fragment does not have the same ability to divide longitudinally.

According to current models of human lung deposition (Yu et al. 1995), fibres with lengths <10 μm and with a diameter range of 0.8 - 2 μm have the maximum probability (~14%) of depositing in the pulmonary region of the lung. Therefore cleavage fragments have the highest probability of lung deposition, while typical

airborne asbestos fibres have a lower probability (~ 5%). The measured width of the fibres produce an over estimate the fibre volume and mass if the fibre is assumed to have a square cross section. Due to the irregular cross-section and preferred orientation on the filter, the actual cross-sectional area is about half the square of the measured width for amphibole asbestos. Therefore a correction factor is needed when calculating fibre respirability and potency if comparisons are made with MMVF's which have near circular cross-sections. This means that amphibole asbestos fibres and mineral fragments above 1 μm width are of concern as they will be more respirable than MMVF 's of the same diameter.

4.3.7 *Conclusions from toxicology for measurement methods*

These differences in fibril size and whether it is a single crystal fibre and an asbestos fibre bundle of crocidolite or anthophyllite are therefore crucial to the whole process of hazard and risk assessment. To adequately measure the hazard it would appear necessary to determine the true potential of the asbestos fibre to divide into fibrils and for the material to break into elongated cleavage fragments. This suggests that a substantial challenge and force is necessary to divide the sample and recover and identify the numbers of fibres produced. The width of the fibres to be included in the analysis of hazard and risk need careful assessment, as it will bias the type of fibre being counted (asbestos or cleavage fragment) as well as having a large effect on the precision of the mass measurement. The animal data also shows that the mass of the fibres is not a accurate measurement of hazard. The number of high potency fibres per unit mass of solid tested, is a far better measure of both hazard and risk. This suggests that the EU screening method based on the ratio of fibre : sample mass by light microscopy will not be a very specific method to measure either the hazard or risk from asbestos in a mineral substance. A reference method based on TEM capable of viewing and identifying fine fibres is essential.

5. SUMMARY OF METHODS DEVELOPED AND THEIR PERFORMANCE IN INTER-LABORATORY COMPARISONS

Three methods were developed during this project:

1. An EU method for identifying asbestos in bulk materials.

2. A semi-quantitative light microscopy screening method to discriminate the mass percentage of possible asbestos in a substance

3. A quantitative TEM reference method for the identification, size distribution, fibre number and mass concentration measurement of asbestos.

All three methods have been developed and documented to meet European (EN) or international (ISO) standard formats and subject to inter-laboratory trials. These appear as annexes 2-4 to this report.

5.1 EU method for identifying asbestos in bulk materials

This method formed an independent but complementary part of the semi - quantitative method (figure 3) and was based on work previously carried to produce MDHS 77 and the EU bulk identification procedure (Burdett, 1996). The identification is based on an initial stereo-microscopic examination at low magnification and selecting representative fibres or grab samples for polarised light microscopy identification at magnifications of X100 - X 500 (figure 4). The draft was first adapted for EU use and then subject to three round of comments. The first to the 4 EU contract laboratories and the second and third to the 24 participating laboratories during 1994 and early 1995. 17 and 20 replies were received from rounds 2 and 3 respectively. Some comments from outside this group were also received, to form a final draft in January 1996 which was used for inter-laboratory trials. A revised final draft was produced at the end of 1997 for the report to DGXI incorporating minor changes and amendments after the laboratory trials. and to interface better with the semi-quantitative method.

The inter-laboratory comparison consisted of two round robins amongst the 24 laboratories who attended the initial meeting in Brussels in June 1992. All samples and replies were prepared and processed by HSL. The performance of the laboratories using this method were compared to a set of UK laboratories using MDHS 77. The details of the samples and scoring appear as appendix 5 and are summarised in table 3.

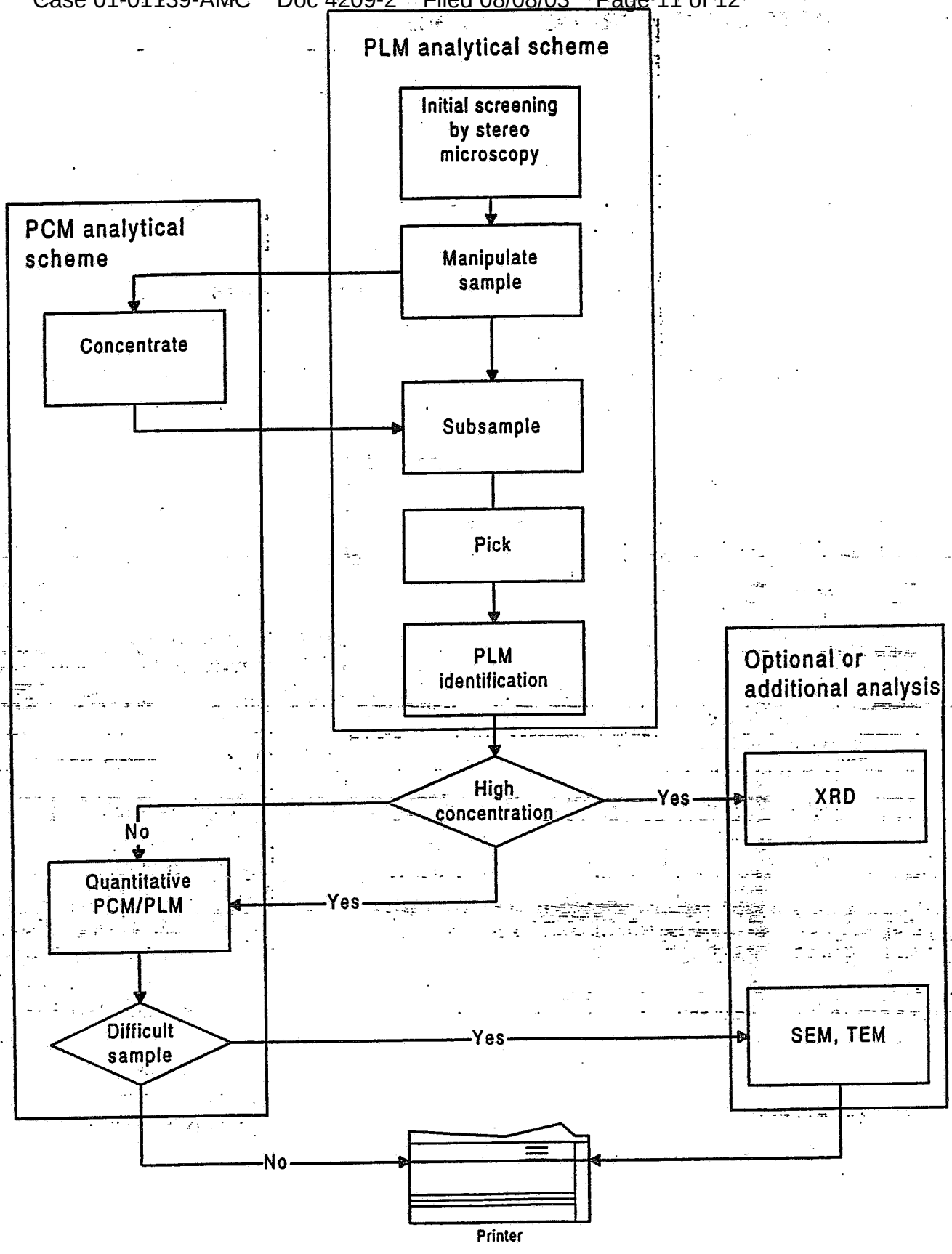
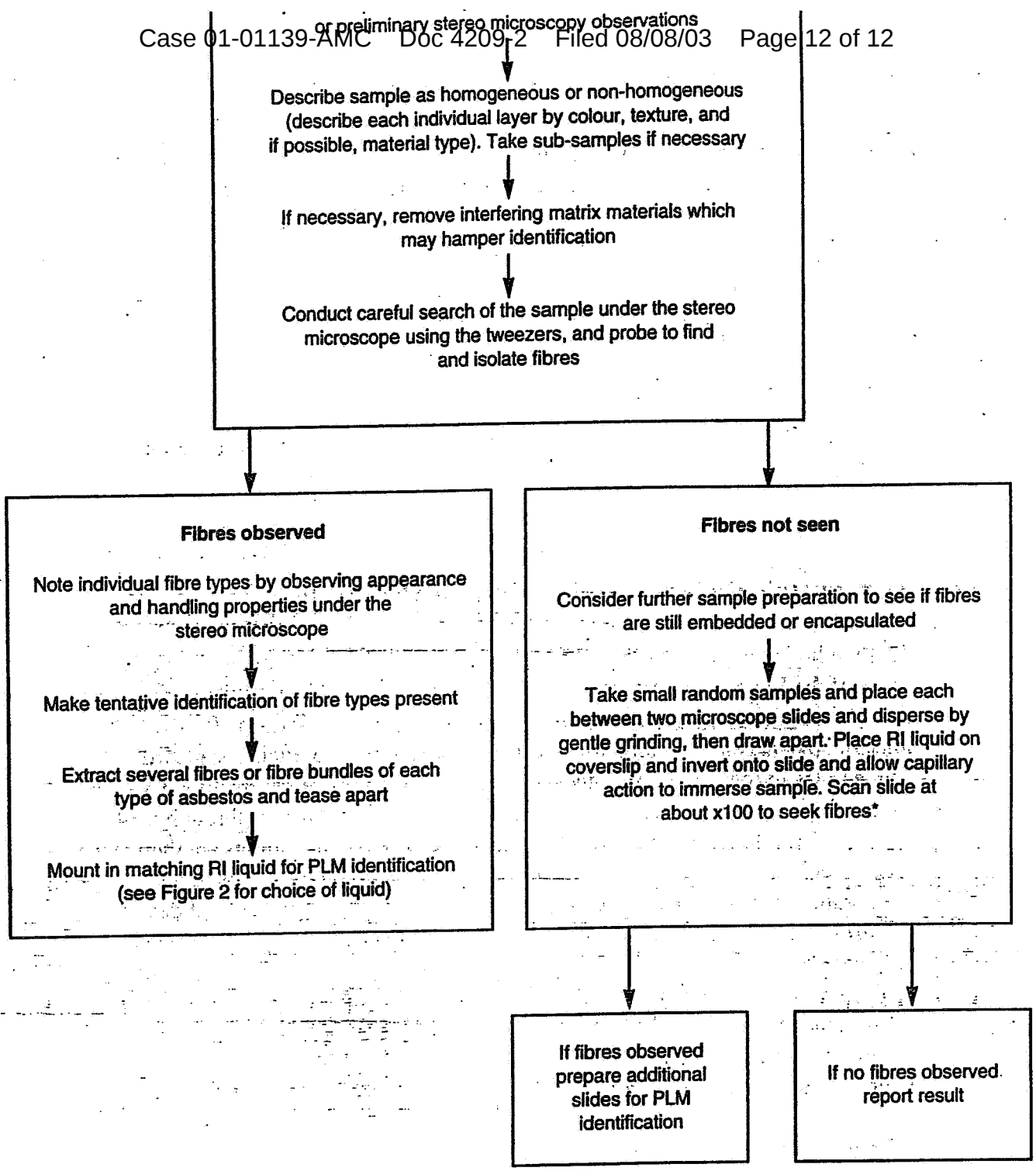


Figure 3: Flow diagram of overall analysis scheme



**Note: Very fine fibres were used in vinyl asbestos tiles and may be present in dusts: thus, higher magnifications may be required to detect asbestos.*

**Note: Search for chrysotile in a liquid to give high contrast (eg water, or RI of 1.67).*

Figure 4: Initial optical microscopy analysis scheme.